Response of anti-SARS-CoV-2 total antibodies to nucleocapsid antigen in COVID-19 patients

Favresse, Julien; Eucher, Christine; Elsen, Marc; Laffineur, Kim; Dogné, Jean-Michel; Douxfils, Jonathan

Published in:
Clinical Chemistry and Laboratory Medicine

DOI:
10.1515/cclm-2020-0962

Publication date:
2020

Document Version
Publisher's PDF, also known as Version of record

Letter to the Editor

Julien Favresse*, Christine Eucher, Marc Elsen, Kim Laffineur, Jean-Michel Dogné and Jonathan Douxfils

Response of anti-SARS-CoV-2 total antibodies to nucleocapsid antigen in COVID-19 patients: a longitudinal study

https://doi.org/10.1515/cclm-2020-0962
Received June 22, 2020; published online xxx

Keywords: COVID-19; kinetics; SARS-CoV-2; serology; symptom onset.

To the Editor,

The detection of anti-SARS-CoV-2 antibodies is valuable to improve the sensitivity of pathogenic diagnosis for COVID-19, to identify convalescent plasma donors, to screen the population to determine the seroprevalence and to assess the persistence of protection in the population or at the individual level [1–3]. A wide range of serology immunoassays have been developed to complement the RT-PCR, with different SARS-CoV-2 antigen targets and formats [4]. Due to the widespread dissemination of these methods and the limited experience with these new assays, it is crucial for laboratories to rigorously validate these methods before broad introduction into routine clinical practice [1, 3, 5].

Recently, the performances of the Elecsys assay have been published. The Elecsys anti-SARS-CoV-2 is an electrochemiluminescent immunoassay (ECLIA) for the *in vitro* qualitative detection of total antibodies, including IgG, to SARS-CoV-2 in human serum and plasma. The assay uses a recombinant protein representing the nucleocapsid antigen for the determination of antibodies against SARS-CoV-2. In our study, all measurements were performed on the cobas® e801 (Roche Diagnostics®). Three independent studies found a specificity ranging from 98.7 to 100% [6–8]. Sensitivities two weeks post-symptoms were 89.4% (n=47) [7], 91.1% (n=79) [6], and 100% (n=18) [8], using the manufacturer's cut-off (i.e., COI ≥ 1.0). In one of these studies, optimization of the cut-off, as determined by ROC curve analyses, was associated with a sensitivity of 95.1% without diminishing the specificity of 100%, suggesting that cut-off optimization may increase the detection rate in the population [6]. Antibody kinetics is a matter of concern, especially because emerging data showed that the persistence of antibodies may last only 2 to 3 months, offering a strong note of caution against the idea of "immunity certificate" [9]. In this study, 40 and 13 percent of asymptomatic and symptomatic patients had antibody levels falling below the threshold for positivity in the early convalescent phase, i.e., eight weeks after discharge from the hospital. However, even low levels of neutralizing antibodies may still be protective, arguing for the use of optimized cut-off to detect those with low antibody levels. The aim of the present study was to assess the longitudinal kinetics of anti-SARS-CoV-2 antibodies since symptom onset, in a large cohort of patients, and by using the manufacturer's cut-off and the optimized cut-off (i.e., COI > 0.165) recently published [6].

This study has been conducted from March 21 to May 25, 2020 at the clinical biology laboratory of the Clinique Saint-Luc Bouge (SLBO, Namur, Belgium). A total of 150 serum samples were obtained from 94 patients confirmed positive to SARS-CoV-2 by RT-PCR. Information on the days since the onset of symptoms was collected from the medical records. The RT-PCR for SARS-CoV-2 determination in respiratory samples (nasopharyngeal swab samples) was performed on a LightCycler® 480 Instrument II (Roche Diagnostics®) using the LightMix® Modular SARS-CoV E-gene set. Blood samples collected from patients into serum-gel tubes (BD Vacutainer® 8.5 mL tubes, Becton Dickinson, New Jersey, USA) or lithium-heparin plasma

***Corresponding author: Julien Favresse**, Department of Laboratory Medicine, Clinique Saint-Luc Bouge, 8 Rue Saint-Luc, Bouge, 5000, Namur, Belgium; Department of Pharmacy, Namur Research Institute for Life Sciences, University of Namur, Namur, Belgium, Phone: +32 81 20 91 44, E-mail: j.favresse@labstluc.be
Christine Eucher, Marc Elsen and Kim Laffineur: Department of Laboratory Medicine, Clinique St-Luc Bouge, Namur, Belgium
Jean-Michel Dogné: Department of Pharmacy, Namur Research Institute for Life Sciences, University of Namur, Namur, Belgium
Jonathan Douxfils: Department of Pharmacy, Namur Research Institute for Life Sciences, University of Namur, Namur, Belgium; Qualiblood sa, Namur, Belgium. https://orcid.org/0000-0002-7644-5298

tubes (BD Vacutainer® 4.0 mL tubes) according to standardized operating procedure and manufacturer recommendations. Samples were centrifuged for 10 min at 1,885×*g* (ACU Modular® Pre Analytics, Roche Diagnostics®). Sera and plasma samples were stored at −20 °C and thawed 1 h at room temperature on the day of the analysis. Re-thawed samples were vortexed before the analysis. Antibody kinetics was evaluated using COI results obtained in all samples using the following time frames (d, days): 0–5 d, 6–8 d, 9–11 d, 12–14 d, 15–17 d, 18–20 d, 21–23 d, 24–30 d, 31–40 d, 41–63 d. Maximum one serum per category per patient was allowed leading to the exclusion of five samples (number of samples included = 145). The mean COI results (and standard errors) were plotted against the different time frames. Smoothing splines with four knots were used to estimate the time kinetics curve. Data analysis was performed using GraphPad Prism® software (version 8.2.1, San Diego, CA, USA). Our study fulfilled the Ethical principles provided by the Declaration of Helsinki.

Figure 1A shows the frequency of reported symptoms. Fever was the most frequent symptom (68.1%), followed by cough (60.4%), fatigue (58.2%), difficulty breathing (45.1%), and muscle aches (31.9%). Less frequency reported symptoms were chest pain (6.6%), sore throat (6.6%), and loss of smell (6.6%). The median number of symptoms per patient was 3 (Figure 1B). Figure 2 reports the antibody kinetics at different days from symptom onset in 145 sera samples from 94 patient and shows the number and percentage of positive test results for each time category, according to the manufacturer's cut-off and the optimized cut-off (>0.165) [6], respectively. Positivity rates prior day 15 were insufficient to recommend the use of this serological assay in clinical practice in this timeframe whatever the cut-off used. The manufacturer's cut-off provided a positivity rate from 0 to 84.6% and the optimized cut-off, from 16.7 to 100%. After 15 days since symptom onset, positivity rates increased from 86.7 to 100% using the manufacturer's cut-off and from 89.5 to 100% using the optimized cut-off (Figure 2).

Current data suggest that seroconversion occurs approximately 7–14 days after symptom onset [1]. In our study, a gradual increase of total antibodies since the onset of COVID-19 symptoms was observed (Figure 2). After ±7 days, the positivity rates in our cohort was around 70% meaning that one could wait few days to improve detection of SARS-CoV-2 antibodies in a broader population. From day 15, and based on the manufacturer's cut-off (i.e., COI ≥ 1.0), eight serum samples were considered negative giving a mean positivity rate of 90.8%. Using the optimized cut-off (i.e., >0.165) [6], the mean positivity rate

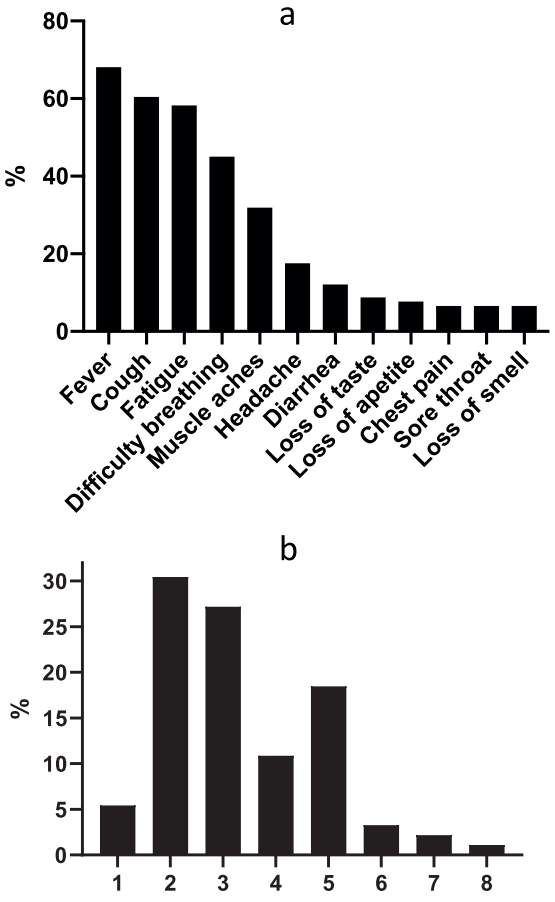
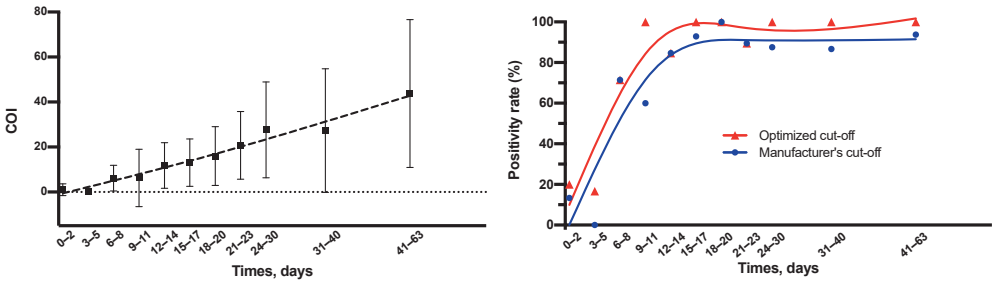


Figure 1: Frequency and number of symptoms in the studied COVID-19 patients. (A) frequency of reported symptoms, (B) number of symptoms per patient.

reaches 97.7%. From day 24, the positivity rate increased to 100% (Figure 2).

Other studies confirmed that early anti-SARS-CoV-2 antibody determination had limited positivity rates. In a study on 37 COVID-19 patients, Padoan et al. observed that 12 days since fever onset were required to reach 100% sensitivity for IgG on the Maglumi 2000 Plus CLIA (chemiluminescent assay) [10]. A lower sensitivity (88%) was however found for IgM and a cut-off refinement was therefore suggested [10]. Tang et al. compared the Abbott IgG, the Euroimmun IgG, and the Elecsys total antibodies SARS-CoV-2 assays in 48 COVID-19 patients [7, 11]. After two weeks since symptom onset, they found sensitivities of 93.8, 85.4, and 89.4%, respectively. The sensitivity before day 14 was higher for the Elecsys assay compare to Abbott and Euroimmun assays [11]. Nevertheless, none of the three assays had sufficient sensitivity to be useful in identifying post-COVID-19 infection before day 14. Montesinos et al. showed equivalent performance of five anti-SARS-CoV-2 assays (Euroimmun Anti-SARS-CoV-2 ELISA IgG, and IgA assays, Maglumi™2019-n-Cov IgG and IgM CLIA assay and



Times since symptom onset	0–2 days	3–5 days	6–8 days	9–11 days	12–14 days	15–17 days	18–20 days	21–23 days	24–30 days	31–40 days	41–63 days
n	15	6	14	10	13	14	7	19	16	15	16
Mean±SD (COI)	1.07±2.62	0.10±0.05	6.17±5.74	6.25±12.80	11.78±10.15	13.09±10.50	15.97±13.05	20.73±15.02	27.60±21.31	27.30±27.52	43.80±32.88
95%CI of mean (COI)	−0.38–2.52	0.04–0.16	2.85–9.48	−2.88–15.39	5.65–17.91	7.01–19.18	3.90–28.04	13.49–27.96	16.24–38.95	12.06–42.54	26.28–61.32
Median (IQR) (COI)	0.079 (0.08–0.12)	0.08 (0.08–0.12)	5.63 (0.09–11.35)	1.40 (0.27–5.89)	15.00 (1.73–19.15)	9.64 (5.12–20.43)	12.50 (3.61–33.00)	20.10 (9.57–33.10)	28.70 (6.42–33.53)	16.90 (6.57–51.30)	37.20 (13.93–78.68)
Minimum-maximum (COI)	0.07–9.50	0.07–0.21	0.08–15.50	0.21–41.90	0.08–29.60	0.70–35.00	3.16–31.34	0.08–51.80	0.17–74.50	0.64–80.40	0.60–90.90
n positive, manufacturer's cut-off (%)	2/15 (13.33%)	0/6 (0%)	10/14 (71.43%)	6/10 (60.00%)	11/13 (84.62%)	13/14 (92.86%)	7/7 (100%)	17/19 (89.47%)	14/16 (87.5%)	13/15 (86.67%)	15/16 (93.75%)
n positive, optimized cut-off (%)	3/15 (20.00%)	1/6 (16.67%)	10/14 (71.43%)	10/10 (100%)	11/13 (84.62%)	14/14 (100%)	7/7 (100%)	17/19 (89.47%)	16/16 (100%)	15/15 (100%)	16/16 (100%)

Figure 2: Descriptive statistics of anti-SARS-CoV-2 total antibodies for the 94 studied patients, subdivided on the basis of each time point and graphical representations of antibody kinetics (COI) and positivity rate (%) according to days since symptom onset. Patients may differ between different time points. The spline curve only provides an estimate of positivity rates but already highlights the need to use an optimized cut-off to improve the performance of the test whatever the time point.

three lateral flow assays) 14 days after the symptoms onset [12]. Overall, sensitivities 15 days after the onset of COVID-19 symptoms varied from 87.5 to 93.93% (n=32–33, depending on the assay considered) and were higher compared to early determinations. Pan et al. found increasing positivity rates of 3.6, 57.1, and 96.8% in early (0–7 days, n=27), intermediate (8–14 days, n=28) and late stage (>15 days, n=31) since symptom onset on a colloidal gold-based immunochromatographic strip assay (Zhuhai Livzon Diagnostic Inc.) in 67 COVID-19 confirmed patients [13]. From 17 days since symptom onset, Long et al. found 100% positivity for IgG (MCLIA kits, Bioscience Co.) in 285 patients with COVID-19 [14]. IgG positivity rates at 11–13 days and 14–16 days since symptom onset were lower with respective rates of 68.6 and 90%, respectively.

In conclusion, we found a continuous antibody increase since symptom onset. We confirmed that a minimum of 2 weeks since symptom onset is needed to increase anti-SARS-CoV-2 detection. The use of an optimized cut-off allowed to increase positivity rates and provides earlier detections. Further studies designed to evaluate long-term antibody kinetics are also needed to address the persistence of the immunity response and the performances of the different assays in detecting potential lower levels of antibodies.

Disclosures

Among the authors, Jonathan Douxfils is chief executive officer and founder of QUALIblood sa and reports personal fees from Diagnostica Stago, Roche, Roche Diagnostics, Daiichi-Sankyo, and Portola, outside the submitted work. Roche Diagnostics generously provided the kits for the validation.

Acknowledgments: We wish to thank the personnel of the Saint-Luc Bouge laboratory for its technical assistance.
Research funding: None declared.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Authors state no conflict of interest.
Ethical approval: The study fulfilled the Ethical principles provided by the Declaration of Helsinki.

References

1. Bohn MK, Lippi G, Horvath A, Sethi S, Koch D, Ferrari M, et al. Molecular, serological, and biochemical diagnosis and monitoring of COVID-19: IFCC taskforce evaluation of the latest evidence. Clin Chem Lab Med 2020;10:1037–52.
2. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis 2020;28:1–22.
3. Farnsworth CW, Anderson NW. SARS-CoV-2 serology: much hype, little data. Clin Chem 2020. [Online ahead of print]. https://doi.org/10.1093/clinchem/hvaa107.
4. Vashist SK. In vitro diagnostic assays for COVID-19: recent advances and emerging trends. Diagnostics (Basel) 2020;10:202.
5. Diamandis P, Prassas I, Diamandis EP. Antibody tests for COVID-19: drawing attention to the importance of analytical specificity. Clin Chem Lab Med 2020;58:1144–45.

6. Favresse J, Eucher C, Elsen M, Marie TH, Dogne JM, Douxfils J. Clinical performance of the Elecsys electrochemiluminescent immunoassay for the detection of SARS-CoV-2 total antibodies. Clin Chem 2020. [Online ahead of print]. https://doi.org/10.1093/clinchem/hvaa131.
7. Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, et al. Clinical performance of the Roche SARS-CoV-2 serologic assay. Clin Chem 2020. [Online ahead of print]. https://doi.org/10.1093/clinchem/hvaa132.
8. Egger M, Bundschuh C, Wiesinger K, Gabriel C, Clodi M, Mueller T, et al. Comparison of the Elecsys(R) Anti-SARS-CoV-2 immunoassay with the EDI enzyme linked immunosorbent assays for the detection of SARS-CoV-2 antibodies in human plasma. Clin Chim Acta 2020;509:18–21.
9. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 2020. [Online ahead of print]. https://doi.org/10.1038/s41591-020-0965-6.
10. Padoan A, Cosma C, Sciacovelli L, Faggian D, Plebani M. Analytical performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics. Clin Chem Lab Med 2020;58:1081–88.
11. Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, et al. Clinical performance of two SARS-CoV-2 serologic assays. Clin Chem 2020. [Online ahead of print]. https://doi.org/10.1093/clinchem/hvaa120.
12. Montesinos I, Gruson D, Kabamba B, Dahma H, Van den Wijngaert S, Reza S, et al. Evaluation of two automated and three rapid lateral flow immunoassays for the detection of anti-SARS-CoV-2 antibodies. J Clin Virol 2020;128:104413.
13. Pan Y, Li X, Yang G, Fan J, Tang Y, Zhao J, et al. Serological immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-19 patients. J Infect 2020;81:e28–32.
14. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020;26:845–8.